vials have been reserved for long-term stability testing under various conditions, including elevated temperatures and humidities, to test the integrity and durability of the packaging system. As packaged for clinical use, 20 ml vials have been dry-filled with 110 mg of ethylene oxide sterilized artesunate, stoppered, and sealed. Stability studies at Knoll have shown at least two years stability for bulk artesunic acid stored under nitrogen @ 25° C.

[0064] The sterilized bulk drug of the invention has been tested and is still undergoing stability studies. The sterilized bulk drug has shown no evidence of degradation for 20 months at 25° C. The stability studies are still ongoing.

[0065] Having generally described this invention, a Further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

Examples

Example 1

GMP Formulation and Packaging

[0066] Upon receipt of the accessible portions of the European Drug Master File (DMF) for artesunic acid from Knoll, the inventors compared their analytical protocols for artesunic acid to those used in the DMF. The DMF method used by Knoll is as follows:

[0067] Validation of an HPLC-Based Assay for AS

[0068] HPLC was performed using the following conditions:

LC system	
Solvent Delivery	Waters 600 Pump System Controller
Injector	Waters 717+ Auto Sampler
Detector	Waters 996 Photo Diode Array (PDA)
Quantitation Software	Empower, Build Number 1154
Method Conditions	
Column	YMC ODS-AQ^2 250 mm Length × 4.6-mm ID, 3 μm
Mobile Phase	35:65 A:B where A = 0.01M potassium dihydrogen phosphate, pH 3.8, and B = Acetonitrile
Flow Rate	1.20 mL/min; pressure~2400 psig
Injection Size	30-μL
Run Time	20 min
Detection	UV @ 205 nm

The reference solutions (n=5 each) were prepared by accurately weighing between 3.472 to 15.977 mg of the reference and dissolving each in 1.00 mL of acetonitrile. A series of 30-μL injections were made to deliver 104.2 to 479.3 μg of reference on column for assay.

[0069] Calculations (Apply to Both Reference and Sample)

[0070] The mass of sample on column (m_x, μg) was calculated using equation one (EQ. 1)

$$m_x = W_x \times (V_1/V_x) \quad \text{Eq. 1}$$

where, W_x is the sampled mass (mg) of the reference or sample (S) as weighed, V_x is the volume of solvent (1.00 mL acetonitrile) used, and V_1 is volume of solution injected (30 μL). An area to mass on column response factor (RF_A) was calculated for the reference standard using equation two (Eq. 2)

$$RF_A = (A_R/m_R) \times (100\%/P_R) \quad \text{Eq. 2}$$

[0071] Where, A_R is the reference peak area, and P_R is the reference purity (>99%)[3]. Sample peak area data was used in equation three (Eq. 3) to calculate the mass (m_s) of the sample,

$$m_s = A_S \times (100\%/RF_A) \quad \text{Eq. 3}$$

where A_s is the sample peak area.

[0072] Duplicating all the experimental conditions used by Knoll, the inventors confirmed the results of its previously validated HPLC assay. Upon validation of the imported Knoll assay, it was adopted as one of the assays to be used by the inventors to confirm the identity of artesunic acid samples and to test the purity of such samples. The major advantage of the Knoll method was lowering the LOD from 2 ug to 0.075 ug on column and decreasing the assay time from 16 minutes to 8 minutes. The major disadvantage is its inability to determine AS in phosphate. Precision, linearity, quantation, and accuracy were comparable for both methods.

[0073] The inventors verified the identity and determined the purity of three samplings of WR256283; BQ38641, (Knoll Lot 2.03). This was the milled sample of the bulk Knoll drug substance used in formulation of the injectable artesunic acid for clinical trials. The three samples were taken to confirm the identity and uniformity of the received material (Sample A from the top of the container, Sample B from the middle of the same container, and Sample C from the bottom of the container). They were compared to a reference sample received Jun. 29, 2001 (WR256283; BP18288) using a number of analytical tests including, but not limited to, Fourier Transform Infrared Spectroscopy, Proton Nuclear Magnetic Resonance Spectroscopy, Elemental Analysis, High Performance Liquid Chromatography, Thermogravimetric Analysis, Residual Solvents by Gas Chromatography, and Inductively Coupled Plasma. The samples were confirmed as being identical samples of artesunic acid. Purity was determined with an HPLC-based assay using the external standard method, with a known reference purity of >99%. HPLC results confirmed sample purity was 99.3 plus or minus 0.3%. Residual solvents in the Knoll material include heptanes (0.09%) and ethyl acetate (0.04%), plus trace amounts (<0.01%) of methanol and ethanol. Lead was not found.

[0074] SRI verified that an ethylene oxide sterilization treatment (4 hours at 102 degrees F.) does not degrade artesunate; the treated material meets USP requirements for sterility. The EtO treated sample was purged with nitrogen to remove residual ethylene oxide. Subsequently, bioburden, bacteriostasis, fungistasis, and endotoxin tests were performed to validate the sterility treatment method. Tests for ethylene oxide derivatives were negative and the residual EtO was found to be well below the FDA recommended levels. Tests for artesunate breakdown products, including dihydroartemisinin, were similarly negative. Results from validated bioburden and LAL tests on sterilized artesunate met USP requirements for sterility and endotoxins. The average chromatographic purity after ethylene oxide treatment was found to be 99.9 plus or minus 0.4% relative to the reference standard. Qualitative and quantitative assay results verified the chemical integrity of the ethylene oxide-treated artesunate. These results establish the time zero data point for future ethylene oxide-treated artesunate stability studies.

[0075] Six thousand dry-filled vials of formulated artesunic acid for clinical use have been packaged. One thousand of the vials have been reserved for long-term stability testing under various conditions, including elevated temperatures and